

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. : 10/591,920 Confirmation No. 5381
Applicants : Yoshito TOBE et al.
Filed : November 22, 2004
Title : MOLECULAR-WIRE TYPE FLUORESCENT CHIRAL
SENSOR
Group Art Unit : 1797
Examiner : David G. Weisz
Customer No. : 28289

SECOND DECLARATION UNDER 35 U.S.C. §1.132

I, Dr. Yoshito Tobe, declare and say as follows:

1. I obtained a Bachelor of Engineering degree from Osaka University in 1974. I obtained a Master of Engineering degree from Osaka University in 1976. I obtained a Ph.D. degree in Engineering (in synthetic chemistry) from Osaka University in 1979.

2. From 1979-1983, I was a Research Associate with the Faculty of Engineering Science at Osaka University. From 1983-1992, I was an Assistant Professor with the Faculty of Engineering Science at Osaka University. From 1992-1998, I was an Associate Professor with the Faculty of Engineering Science at Osaka University. From 1998 to the present, I have been a Professor in the Graduate School of Engineering Science at Osaka University.

3. My primary work has included creating new materials which have novel structures, and to reveal the properties based upon structural features, including projects relating to Extended pi-Electron Systems and Precise Molecular Recognition.

4. I am familiar with the subject matter of U.S. Application Serial No. 10/590,920, of which I am an inventor. I am familiar with the rejection of the specification and claims 1-5 and 7-8 under 35 U.S.C. §112, first paragraph for alleged lack of disclosure for a

linking conjugated system other than ethynylene and lack of enablement. Also, I am familiar with the rejection of claims 1-3, 5 and 7-8 under 35 U.S.C. §103(a) as being unpatentable over Naemura et al., "Temperature Dependent Reversal of Enantiomer Selectivity in the Complexation of Optically Active Phenolic Crown Ethers with Chiral Amines", *Chem. Commun.* (1996) 2749-2750 in view of Kim et al., "Ion-Specific Aggregation in Conjugated Polymers: Highly Sensitive and Selective Fluorescent Ion Chemosensors", *Angew. Chem. Int. Ed.* (2000) 39, No. 21, 3868-3872. In response to this rejection, I set forth my opinion and comments below.

5. In my opinion, the components of fluorescent molecular wire (that is, the optically active substituent, polymer main chain and a linked conjugated system) of the claimed invention are fully supported by the specification in view of the technological knowledge at the time of filing the present application.

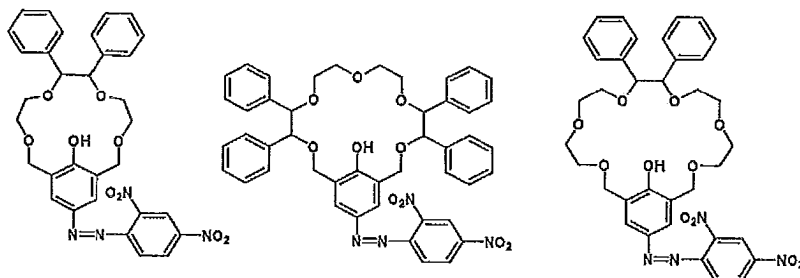
Many portions of the compounds included in formula (I) of claim 1 are recognized to have asymmetry recognition ability. Generally, it is apparent to one skilled in the art that an optically active host compound forms each different diastereomer complex with guest compounds composed of a chiral pair of enantiomers. On the other hand, it is well known that host compounds having many ether oxygens as electron donors and hydroxyl groups as hydrogen donors at the guest capturing position can form complexes with guest compounds.

Therefore, when a compound, such as formula (I) of claim 1, has both a chiral structure and electron donors or hydrogen donors that act as a host, can exhibit so large asymmetry recognition ability. Even if the compound does not have a ring structure (for example, reference 9 below), the asymmetry recognition ability is so large that guest enantiomers can be differentiated by ultraviolet-visible absorption spectrometer. Generally if a compound has a ring structure, a rate of complexation and asymmetry recognition ability becomes large.

Optically active portions of compounds in the structural formula (I) of claim 1 have asymmetry recognition ability, as shown by the following References 1-9:

Reference 1: T. Kaneda et al., J. Am. Chem. Soc. 1989, Vol. 111, pp. 742-743

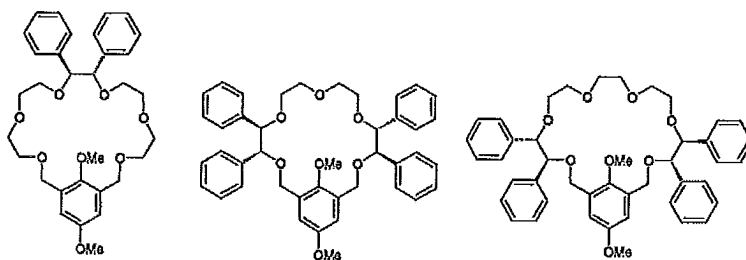
Reference 1 discloses 3 structures of chiral azophenolic acerands at page 742.



It is described that these compounds can form complexes and exhibit enantiomeric amine-selective coloration (pages 742 and 743). It is understood that crown ethers of various sizes can form complexes.

Reference 2: M. Sawada et al., J. Am. Chem. Soc. 1993, Vol. 115, pp. 7381-7388

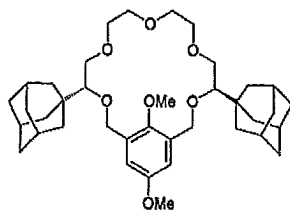
At page 7383 of the reference 2, the structures of chiral crown ethers 10 and 16 are disclosed.



Further in the abstract in page 7381, it is described that the enantioselective host-guest complexation using an ammonium ion compound as a guest was demonstrated. Therefore, a crown ether host can form complexes not only with primary amines but also ammonium salts.

Reference 3: K. Naemura et al., Tetrahedron: Asymmetry 1994, Vol. 5, pp. 1549-1558

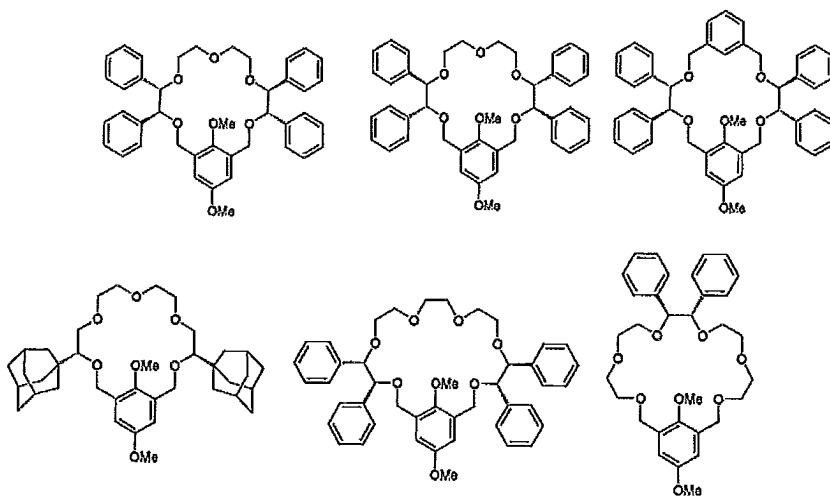
At page 1552 of the reference 3, a structure of crown ether (*S,S*) - (+) - 18 having the adamantyl group is disclosed.



It is disclosed that crown ethers formed a complex with methionine methyl ester perchlorate. It is apparent that even if a substituent in the chiral center of a crown ether is not an aryl group such as phenyl but an aliphatic group such as an adamantyl group, can form complexes.

Reference 4: M. Sawada et al., J. Chem. Soc., Chem. Commun. 1994, pp. 2497-2498

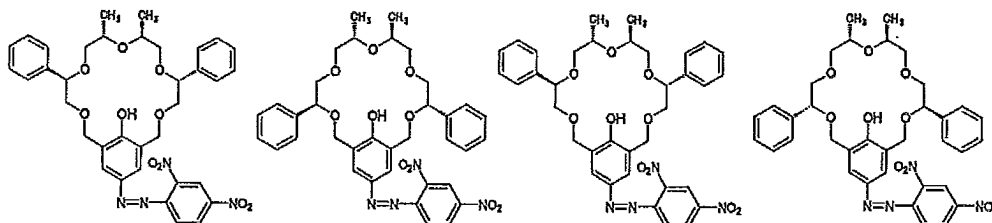
At page 2497 of Reference 4, there are described various host compounds:



It is described that enantioselectivity and complexation ability of these compounds are different from each other, however, these compounds can form complexes. See Table 1 at page 2497. Association constants (K) are shown in the left column, line 15 from the bottom of page 2498.

Reference 5: K. Naemura et al., Tetrahedron: Asymmetry 1997, Vol. 8, pp. 19-22

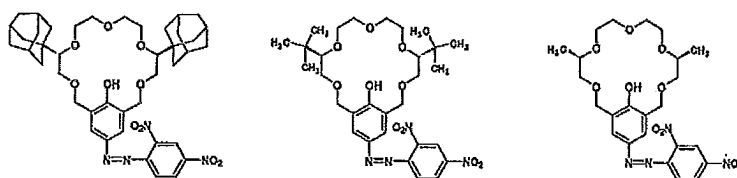
At page 20 of Reference 5, four host compounds in which substituents in the chiral center of crown ethers are placed differently from each other are disclosed:



It is described that even though there are differences in enantioselectivity due to the position of the substituent, these compounds can form complexes.

Reference 6: K. Hirose et al., J. Chem. Soc., Perkin Trans. 1997, Vol. 2, pp. 1649-1657

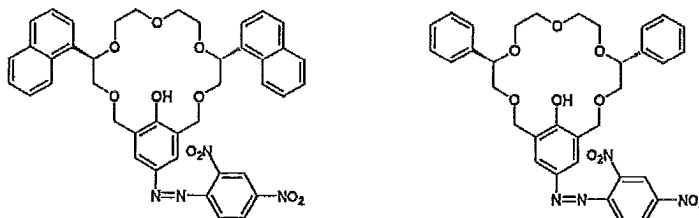
At page 1649 of reference 6, three host compounds having aliphatic groups as substituents in the chiral center of crown ethers are disclosed:



It is described that these compounds can form complexes. See abstract on page 1649 and Table 1 on page 1652.

Reference 7: K. Naemura et al., Tetrahedron: Asymmetry 1998, Vol. 9, pp. 563-574

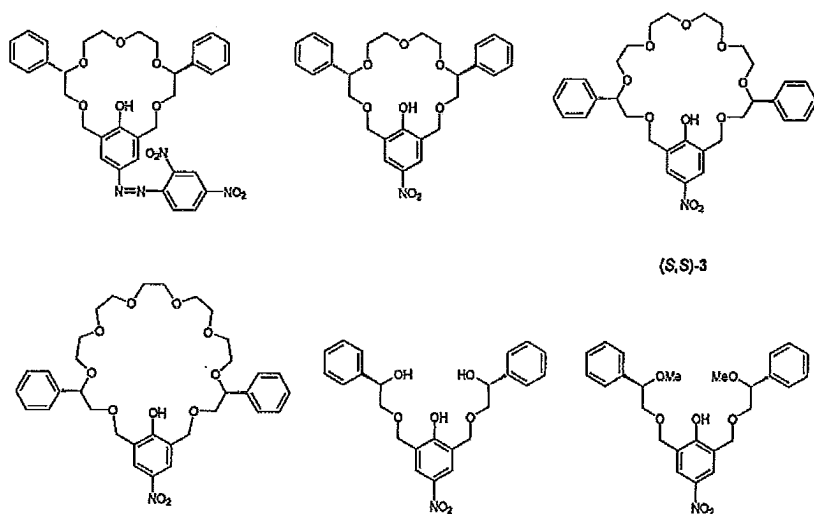
At page 564 of Reference 7, crown ethers having condensed rings as substituents are disclosed:



It is described in the abstract at page 563 that the compounds can form complexes with amines.

Reference 8: K. Hirose et al., Tetrahedron: Asymmetry 2003, Vol. 14, pp. 555-566

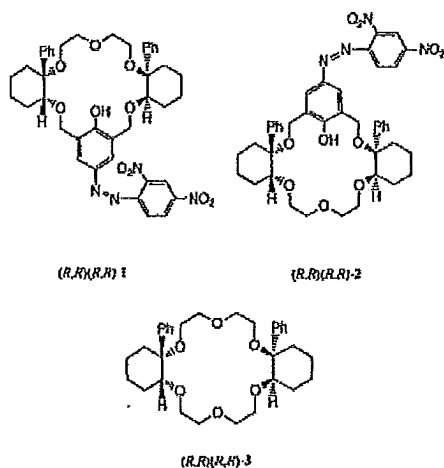
At page 555 of Reference 8, crown ethers having various sizes and podand compounds that do not have ring structures.



It is described in the abstract in page 555 that these podand compounds can have binding ability with secondary amines and a moderate level of chiral recognition ability.

Reference 9: K. Naemura et al., J. Chem. Soc., Perkin Trans. 1996, Vol. 1, pp. 383-388.

At page 383 of Reference 9, host compounds in which a cyclohexane subunit is incorporated into crown ethers so that the chiral center of crown ethers becomes a quaternary carbon atom are disclosed:



In the abstract in page 383, it is described that these compounds have enantioselectivity.

As described above, many compounds are confirmed to have enantioselectivity/complexation ability. These optically active portions of compounds can form a portion of the compound of formula (I) of claim 1. Therefore, Applicants have provided evidence that the substituents of the formula (I) actually have asymmetry recognition ability (enantioselectivity/complexation ability) as shown in the above references.

Evidence that those skilled in the art would recognize easily that the claimed compound can be a sensor for the complexation will now be discussed. That is, between a substituent that forms a complex with a substrate and the portion that is conjugatively-linked to the substituent, information of the complexation can be reflected in the change of electronic and/or optical properties such as coloration and fluorescence, which can be precisely detected by electronic or emission spectra.

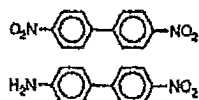
As seen from claim 1, an optically active substituent that forms complexes with optically active amines as substrates should be conjugatively-linked to the polymer main chain that can emit fluorescence. Therefore, a spacer is not always necessary for the invention of claim 1.

First, evidence regarding the effect of conjugation between vinylene groups and ethynylene groups will be discussed.

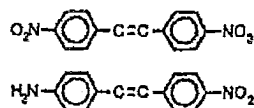
Reference 10: P. P. Shorygin et al., Russian Chemical Bulletin (Translation of Izvestiya Akademii Nauk, Seriya Khimicheskaya) 1998, Vol. 47(2), pp. 297-302.

Reference 10 discloses the change of electronic spectrum compared between the case where two nitrobenzenes are linked together and the case where nitrobenzene (electron acceptor) and benzenamine (electron donor) are linked together in the following manner:

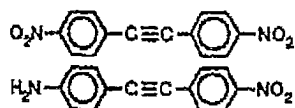
1. directly linked;



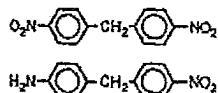
2. linked via vinyl group;



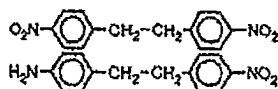
3. linked via ethynylene group;



4. linked via methylene group;



5. linked via ethylene group;

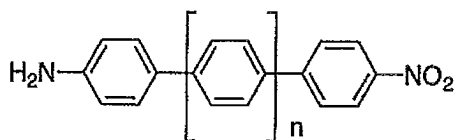


Comparing 1, 2 and 3 with 4 and 5, the shift of absorption band from nitrobenzene for 1, 2 and 3 is larger than that for 4 and 5. This is because the conjugation effect of 1, 2 and 3 is larger than that of 4 and 5. Further, in all the cases, due to the charge transfer between nitrobenzene and benzenamine, a large shift of absorption band is observed compared with the case where two nitrobenzenes are linked together. This is evidence that nitrobenzene and benzenamine are conjugated.

Reference 11: R. Koch et al., J. Phys. Org. Chem. 2008, Vol. 21, pp. 954-962.

Reference 11 provides evidence for the conjugation effect of phenylene groups or arylene groups.

Reference 11 discloses optical properties in a case where plural phenylene groups are introduced between nitrobenzene and benzenamine.

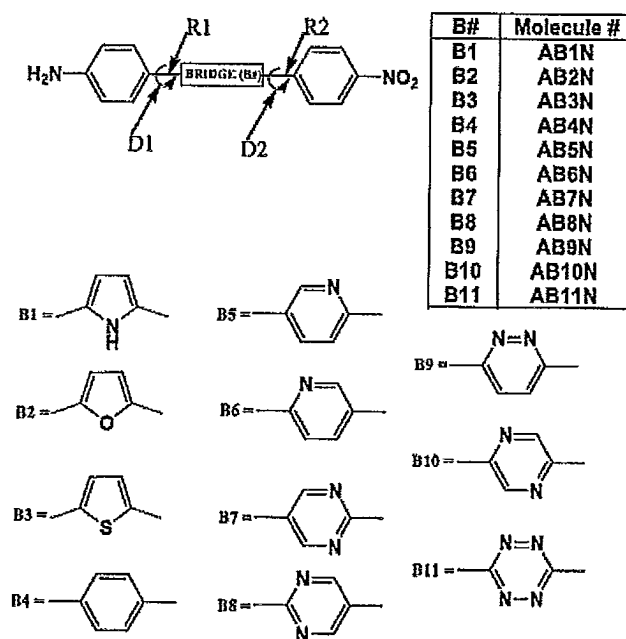


It is shown that the effect of conjugation is satisfactorily large and the optical properties changes even if plural phenylene groups are introduced between nitrobenzene and benzenamine.

Reference 12: S. Sitha et al., J. Mol. Str.: THEOCHEM 2006, Vol. 761, pp. 31-38.

Reference 12 discloses electron transfer energy in a case where arylene groups are introduced between nitrobenzene and benzenamine. It is reported that in any case, conjugation effects appear and electronic state changes largely.

The followings are examples to show that those skilled in the art would recognize easily that the claimed compounds can be a sensor by complexation.

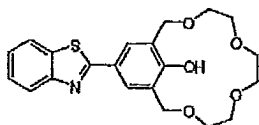


Reference 13: K. Nakashima et al, Bull. Chem. Soc. Jpn. 1987, Vol. 60, pp. 3219-3223.

Reference 13 shows conjugation when crown ether substituents that can form complexes with substrates can directly bind to fluorescent benzothiazoryl groups (without spacer).

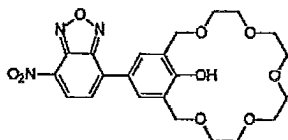
Reference 14: I. Tanigawa et al., Tetrahedron Lett. 1984, Vol. 25, pp. 5327-5330

Reference 14 shows conjugation when crown ether substituents that can form complexes with substrates can directly bind to naphthothiazoryl groups. These show that the complexation can be clearly reflected as a change of fluorescence.



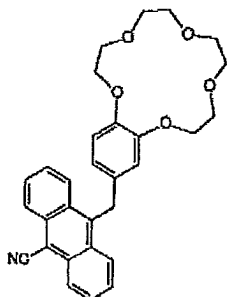
Reference 15: H. Forrest et al., Talanta 1989, Vol. 36, pp. 335-340.

Reference 15 shows conjugation when crown ether substituents that can form complexes with substrates can directly bind to fluorescent groups. These show that the complexation can be clearly reflected as a change of fluorescence emission.



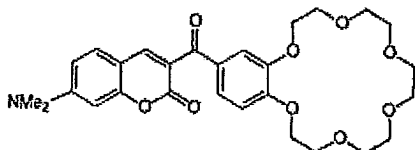
Reference 16: A. P. de Silva et al., J. Chem. Soc., Chem. Commun. 1989, pp.1183

Reference 16 shows that even the conjugation called "homoconjugation" via methylene chain can be a sensor that detects a spectral change as a signal.



Reference 17: R. Crossley et al., J. Chem. Soc., Perkin Trans. 1994, Vol. 2, pp.513.

Reference 17 shows the fluorescence spectral change of a compound that is bound to fluorescent substituents via carbonyl group.



Further, if the compound is conjugated with the chromophore, it is used as a coloration sensor. For example, in a compound of the aforementioned Reference 1, an optically active substituent that can form a complex with a substrate is bound to 2,4-dinitrophenyl group via azo group (-N=N-) to form conjugation. This shows that the complexation can be reflected as a color change.

Further, in the examples of the present specification, optically active substituents that form complexes with substrates are linked to fluorescent polymer main chains so that they are conjugated via ethynyl group. It is known that the complexation can be reflected in the change of electronic spectrum by linking to phenyl groups via ethynyl group, and the compound can be a sensor in examples other than the examples of the present specification (Japanese Laid-Open Patent Publication No. 2004-75624, previously submitted in an Information Disclosure Statement).

As mentioned above, in my opinion those skilled in the art would recognize that between a substituent that forms a complex with a substrate and the portion that is conjugatively-linked to the substituent, information of the complexation can be reflected in the change of electronic spectrum and emission spectrum such as coloration and emission, which enables the compound to be a sensor.

6. The Office Action alleges that one of ordinary skill in the art would choose to link the optically active substituent of Naemura et al. to a conjugated polymer main chain of Kim et al. in order to provide a chemosensor with enhanced sensitivity. However, the combination of the molecular designs of Naemura et al. and Kim et al. are inconsistent because they are individual designs with different specific mechanisms. The design of Naemura et al. is based on thermodynamic equilibrium for non-aggregated homogeneous system. In contrast, the design of Kim et al. is valid for aggregation system based on energy transfer mechanism. Even if the designs of Naemura et al. and Kim et al. could be combined, it was unclear whether the combination was aimed to increase the performance of Naemura et al.'s homogeneous system or that of Kim et al.'s aggregation system. One skilled in the art would understand that aggregation and non-aggregation systems should be independent. Therefore, the motivation to combine the concepts of the molecular designs of Naemura and Kim cannot be rationalized. The combination could not be a correct molecular design before our patent application.

Further, the Office Action alleged that enhanced sensitivity is obtained by the combination of the molecular designs of Naemura et al. and Kim et al. However, it is required for the chiral sensor of the present invention to have not only a high sensitivity but also a high enantioselectivity. Combination of the molecular designs of Naemura et al. and Kim et al. is not a correct motivation for those skilled in the art who expect to satisfy these requirements. The increase of asymmetry recognition ability of the present invention would not be expected even if the designs of Naemura et al. and Kim et al. were combined, and therefore, in my opinion the invention of present claim 1 is significant and inventive.

7. In Naemura et al., for sensing the enantiomer selectivity, the absorption of light, which is proportional to the rate of the complexation of crown ether with a chiral amine, is measured. In Kim et al., for sensing the ion, the aggregation phenomenon of the host molecules is measured as the absorbance or the fluorescence quenching, but it cannot be expected what is proportional to the aggregation. On the other hand, in the present invention, the quenching behavior in emission of the host polymer molecule is measured for sensing the enantiomer selectivity, and the quenching behavior is not associated with the aggregation of the host

polymer molecule. In my opinion, one having ordinary skill in the art can easily understand that the absorption phenomenon, the aggregation phenomenon, and the quenching phenomenon are different. Thus, the sensing mechanisms in Naemura et al., Kim et al., and the present invention are completely different from each other.

The Office Action asserted that there is motivation to combine the molecular structure capable of realizing the absorption of light which is proportional to the complexation rate (i.e., the crown ether of Naemura et al.) with the molecular structure capable of inducing the aggregation phenomenon of sensor molecules (i.e., the polymer of Kim et al.), thereby designing a chiral sensor in which the absorption of light, which is proportional to the complexation, or the aggregation phenomenon is not utilized for sensing. However, the Office Action does not explain why one having ordinary skill in the art has motivation to combine such different sensing mechanisms (i.e., the absorption of light and the aggregation phenomenon) so as to sense the chirality by using further different sensing mechanism, that is, the quenching in emission of the fluorescent molecular wire.


In my opinion, in order to achieve a high sensitivity that could not have been attained by conventional low molecular weight sensors, one having ordinary skill in the art would not have reasonably been expected to combine the sensing mechanisms of Naemura et al. and Kim et al. In general, when the sensing method with a high sensitivity is employed, the sensing has to be performed in the condition of low concentration of the target or sensor molecule, so that the complexation rate tends to be lowered. Therefore, in my opinion, one having ordinary skill in the art does not design a molecular sensor with a high sensitivity based on the sensing method utilizing the absorption and emission phenomena which are proportional to the complexation rate. That is, there is no motivation to combine the molecular structure of Naemura et al. with polymer of Kim et al.

Furthermore, as seen from claim 8, the fluorescent molecular wire of the present invention has also an improved asymmetry recognition ability compared to a monomeric compound having the same structure as the optically active substituent. To this day, there is no generally accepted theory for the improved effect of the asymmetry recognition ability. Therefore, in my opinion, the fluorescent molecular wire of the present invention would not have

been obvious over Naemura et al. and Kim et al. This feature of the present invention can be explained with reference to Fig. 4 of the present specification (see Example 18 on pages 36 to 37).

In particular, the features of the present invention recited in claim 8 are (i) enhancement of detection sensitivity for a substrate to be detected, (ii) achievement of signal conversion to increase selectivity of chiral sensor at the chiral recognition site compared to the corresponding monomer and to amplify the increased asymmetry selectivity, and (iii) simultaneous achievement of (i) and (ii). In view of the technical level at the filing date of the present invention, in my opinion, it would not have been easy to achieve even the feature (i), and even more the feature (ii).

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Dr. Yoshito Tobe
Date April 26, 2010